GENETIC STOCK

Registration of BN611, AN612, BN612, and RN613 Sorghum Genetic Stocks with Stacked *bmr*-6 and *bmr*-12 Genes

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ABSTRACT

Four sorghum [*Sorghum bicolor* (L.) Moench] genetic stocks, BN611 (Reg. No. GS-140, PI 652943), AN612 (Reg. No. GS-141, PI 652944), BN612 (Reg. No. GS-142, PI 652945), and RN613 (Reg. No. GS-143, PI 652946), with stacked brown midrib genes *bmr*-6 and *bmr*-12 were developed jointly by the USDA-ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, and were released in July 2006. The genetic stocks BN611, AN612 and BN612, and RN613 were tested as Atlas *bmr*-6 *bmr*-12, Wheatland *bmr*-6 *bmr*-12, and RTx430 *bmr*-6 *bmr*-12, respectively. Release of these genetic stocks makes stacked brown midrib genes, reported to reduce the activity of two enzymes important in lignin synthesis, cinnamyl alcohol dehydrogenase (*bmr*-6) and caffeic acid O-methyl transferase (*bmr*-12), available in a common forage sorghum, a common grain sorghum seed parent, and a common grain sorghum pollen parent background. The genetic stocks have immediate application for basic research involving lignin synthesis.

Four sorghum [*Sorghum bicolor* (L.) Moench] genetic stocks, BN611 (Reg. No. GS-140, PI 652943), AN612 (Reg. No. GS-141, PI 652944), BN612 (Reg. No. GS-142, PI 652945), and RN613 (Reg. No. GS-143, PI 652946), with stacked brown midrib genes *bmr*-6 and *bmr*-12 were developed jointly by the USDA-ARS and the Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, and were released in July 2006. The genetic stocks BN611, AN612 and BN612, and RN613 were tested as Atlas *bmr*-6 *bmr*-12, Wheatland *bmr*-6 *bmr*-12, and RTx430 *bmr*-6 *bmr*-12, respectively.

An important source of fodder for feed, sorghum is being targeted for use as a bioenergy feedstock and is becoming increasingly important as a model species for basic research. Some qualities that make sorghum ideal for use as a model species are its C_4 metabolism, ability to be grown as an annual, and its relatively small, diploid genome (Sarath et al., 2008). The most successful approach to genetic modification of sorghum cell wall chemistry for feed and bioenergy applications has been through incorporation of brown midrib genes, which are associated with reduced lignin and altered lignin chemical composition (Bucholtz et al., 1980; Porter et al., 1978).

Sorghum has at least three different brown midrib loci (Bittinger et al., 1981). Nineteen independently occurring brown midrib mutants were originally described (Porter et al., 1978). Three of those 19 mutants, *bmr*-6, *bmr*-12, and *bmr*-18, were selected by Porter et al. (1978) for further evaluation and have subsequently been more thoroughly characterized. The mutant *bmr*-6 reduced activity of cinnamyl alcohol dehydrogenase (CAD) (Bucholtz et al., 1980), and the allelic (Bittinger et al., 1981) *bmr*-12 and *bmr*-18 genes reduced caffeic acid O-methyl transferase (COMT) activity (Bout and Vermerris, 2003). Our laboratory previously developed and released sets of sorghum lines near-isogenic for *bmr*-6 and *bmr*-12 (Pedersen et al., 2006a,b,c) and used them to investigate effects of these individual genes on morphology, cell wall chemistry, and yield in multiple genetic backgrounds (Oliver et al.,

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Abbreviations: ADF, acid detergent fiber; ADL, acid detergent lignin; CAD, cinnamyl alcohol dehydrogenase; COMT, caffeic acid O-methyl transferase; NDF, neutral detergent fiber.

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2005a,b). This work demonstrated that genetic background had a significant effect on the phenotypic properties of both *bmr* genes.

Because the *bmr*-6 and *bmr*-12 genes affect different enzymes in lignin synthesis, it is reasonable to hypothesize that their combined effect could result in even greater reduction of lignin content and in greater changes in other cell wall–related traits. However, the genetic resources were unavailable to test this hypothesis. We therefore undertook the development of lines with stacked *bmr*-6 and *bmr*-12 genes near-isogenic to a subset of our previously released lines near-isogenic for the individual *bmr*-6 and *bmr*-12 genes.

The pedigrees of the resultant lines BN611, BN612, and RN613 are 00-2051 (a sister line of N598, PI 639702, synonym = Atlas *bmr*-6, Pedersen et al., 2006b)/00-2172 (a sister-line of 'Atlas *bmr*-12', PI 636763, Pedersen et al., 2006a); 00-1754 (a sister-line of N599, PI 639709, synonym = Wheatland *bmr*-6, Pedersen et al., 2006c)/00-1644 (a sister-line of N600, PI 639710, synonym = Wheatland *bmr*-12, Pedersen et al., 2006c); and 00-1677 (a male-sterile (ms_3ms_3) sister-line of N609, PI 639719, synonym = RTx430 *bmr*-6, Pedersen et al., 2006c)/00-2161 (a sister-line of N610, PI 639720, synonym = RTx430 *bmr*-12, Pedersen et al., 2006c), respectively.

Release of BN611, AN612, BN612, and RN613 makes stacked brown midrib genes reported to reduce the activity of two enzymes important in lignin synthesis, CAD (*bmr*-6) and COMT (*bmr*-12), available in a common forage sorghum, a common grain sorghum seed parent, and a common grain sorghum pollen parent background.

Methods

Development of BN611, AN612, BN612, and RN613

The genetic stocks BN611, AN612, BN612, and RN613 were developed using the pedigree method. They are F_3 or F_4 selections from crosses between sister lines of N598 and 'Atlas *bmr*-12', N599 and N600, and a male-sterile (ms_3ms_3) sister-line N609 and N610, respectively. Crossing between the RTx430 (Miller, 1984) derivatives was facilitated by the use of the nuclear male-sterility gene ms_3. Hand emasculations were used to make the crosses between the 'Atlas' (NSL 3986; Brown et al., 1936) and 'Wheatland' (CIso 918; Brown et al., 1936) derivatives. Selection of individuals homozygous for both *bmr*-6 and *bmr*-12 was accomplished in the F_3 generation for the Atlas derivative, and in the in F_4 generation for the Wheatland and RTx430 derivatives by crossing individual plants to both AN599 (AWheatland *bmr*-6) and AN600 (AWheatland *bmr*-12) and selecting those that produced only the brown midrib phenotype in the resulting progeny. BN612 (Wheatland *bmr*-6 *bmr*-12) was male-sterilized in A_1 cytoplasm by crossing to AN599 (AWheatland *bmr*-6) followed by four backcrosses to BN612 with homozygosity for *bmr*-12 selected for using progeny testing for the brown midrib trait as above.

Confirmation of Fertility Restoration and Presence of *bmr*-6 and *bmr*-12 Genes

The following test crosses were made to confirm fertility restoration and stability of the *bmr* genes when stacked: AN599/BN611, AN599/BN612, AN599/RN613, AN600/BN611, AN600/BN612, AN600/RN613, AN612/BN599, AN612/BN600, and AN612/BWheatland. Approximately 30 F_1 seedlings from each cross were visually examined to determine midrib color in the greenhouse. Six of these F_1 progeny were grown to maturity with inflorescences protected with pollinating bags and examined for presence or absence of seed set.

Field Trials

Field trials comparing BN611, BN612, and RN613 with their wild-type progenitors (Atlas, Wheatland, and RTx430, respectively) and counterparts near-isogenic for *bmr*-6 and *bmr*-12 (Atlas *bmr*-12 and N598, N599 and N600, N609 and N610, respectively) were conducted at the University of Nebraska Field Laboratories at Ithaca, NE, and at Lincoln, NE.

Atlas derivative trials were planted 20 May 2004 at Ithaca and 18 May 2005 at Lincoln. Plots consisted of three 7.6-m rows spaced 76 cm apart. The experimental design was a randomized complete block with five replications in each environment. Nitrogen fertilizer was applied before planting at both locations at 157 kg ha^{-1}. At Lincoln, 3.36 kg ha^{-1} propachlor [2-chloro-*N*-(1-methylethyl)-*N*-phenylacetamide] and 1.1 kg ha^{-1} of atrazine [6-chloro-*n*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4,diamine] were applied immediately after planting for weed control. No supplemental irrigation was applied. At Ithaca, atrazine was applied at 2.2 kg ha^{-1} immediately after planting, followed by an application of quinclorac (3,7-dichloro-8-quinolinecarboxylic acid) and atrazine at 0.37 kg ha^{-1} and 1.1 kg ha^{-1}, respectively, approximately 14 d postemergence. Supplemental irrigation (2.5 cm) was applied at Ithaca via overhead sprinklers on 3, 12, and 19 Aug. 2004. Emergence was determined by counting the number of seedlings in each row approximately 4 wk postplanting. Days to flowering was recorded at 50% anthesis. Height was measured to the top of the mature panicle before harvest. Total biomass yield was determined by harvesting the middle row of each plot using a commercial silage cutter modified for small plot use (Pedersen and Moore, 1995). Grain-free forage samples for laboratory analyses were collected by removing all panicles from one of the outside rows of each plot and harvesting in a similar manner. Subsamples were collected and oven dried (60°C) to determine dry matter for calculation of plot dry matter yields. Ithaca plots were harvested 24 Sept. 2004, and the Lincoln plots were harvested 29 Sept. 2005. All plots were at hard dough or were fully mature.

The Wheatland and RTx430 derivative trials were planted 21 May 2003 at Lincoln and 20 May 2004 at Ithaca. Plots consisted of two 7.6-m rows spaced 76 cm apart. Wheatland and RTx430 derivatives were planted in blocks to minimize border effects. The experimental design was a split plot with lines being whole plots and genotype being

subplots, and four replications in each environment. Cultural practices were the same as described above. Panicles were hand-harvested at maturity; residue was then harvested and dried as described above. Panicles were dried to a uniform 14% grain moisture content and subsequently threshed to determine grain yield. Grain and residue were harvested 1 Oct. 2003 at Lincoln and 6 Oct. 2004 at Ithaca.

Laboratory Analyses

Forage and residue samples were prepared for chemical analyses by grinding in a Wiley mill (2-mm screen; Arthur H. Thomas Co., Philadelphia, PA) and were analyzed sequentially for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) content using an ANKOM 200 fiber analyzer (ANKOM Tech. Corp., Fairport, NY) (Vogel et al., 1999).

Statistical Analyses

The data were analyzed using SAS software (SAS Institute, 2000–2004). Environments and replications were considered random. Least square means were calculated using PROC MIXED, and differences of least square means tested for significance using the PDIFF option of PROC MIXED.

Characteristics

BN611 resembles Atlas with white caryopses color, no tannin-containing testa in the grain, normal and white endosperm, awnless florets, purple necrotic lesion color, and juicy culms, and it does not restore fertility in A_1 cytoplasm. Averaged over environments, BN611 was 4 d later in maturity (83 d vs. 79 d) and 64 cm shorter in height (214 cm vs. 278 cm) than Atlas (Table 1). Seedling emergence of BN611 was slightly higher than for Atlas. Biomass yield of BN611 was significantly lower (10.5 t ha^{-1} vs. 17.7 t ha^{-1}) compared with Atlas. Neutral detergent fiber and ADF of BN611 and Atlas were not significantly different. Acid detergent lignin was significantly lower in BN611 (35 g kg^{-1}) compared with N598 (Atlas *bmr*-6, 45 g kg^{-1}) and Atlas *bmr*-12 (41 g kg^{-1}), which were both significantly lower in ADL content than wild-type Atlas (54 g kg^{-1}). Given that both single mutants show decreases in ADF compared with wild-type Atlas, the lack of further reduction in ADF content in the double mutant BN611 might at first glance appear surprising. However, this is readily explained by equivalent NDF content of BN611 and Atlas and the larger decrease in ADL in BN611 compared with the decrease observed between N598 and Atlas *bmr*-12, and wild-type Atlas. Complete comparisons of BN611 with N598 (Atlas *bmr*-6), Atlas *bmr*-12, and wild-type Atlas are shown in Table 1.

BN612 resembles Wheatland with red caryopses color, no tannin-containing testa in the grain, normal and white endosperm, awnless florets, purple necrotic lesion color, and juicy culms, and it does not restore fertility in A_1 cytoplasm. Averaged over environments, BN612 was 4 d later in maturity (80 d vs. 76 d) and 6 cm shorter in height (98 cm vs. 104 cm) than Wheatland (Table 2). Seedling emergence of BN612 was slightly lower than for Wheatland. Grain yield of BN612 was equivalent (5.6 t ha^{-1} vs. 6.3 t ha^{-1}) to that of Wheatland, while residue yield of BN612 was significantly lower (4.6 t ha^{-1} vs. 5.2 t ha^{-1}) compared with Wheatland. Neutral detergent fiber and ADF of BN612 were slightly lower than for Wheatland. Acid detergent lignin was significantly lower in BN612 (47 g kg^{-1}) compared with Wheatland (64 g kg^{-1}), but it was not significantly different from N599 (Wheatland *bmr*-6, 55 g kg^{-1}) or N600 (Wheatland *bmr*-12, 48 g kg^{-1}). Complete comparisons of BN612 with N599 (Wheatland *bmr*-6), N600 (Wheatland *bmr*-12), and wild-type Wheatland are shown in Table 2.

RN613 resembles RTx430 with white caryopses color, no tannin-containing testa in the grain, yellow endosperm, awnless florets, purple necrotic lesion color, and juicy culms, and it restores fertility in A_1 cytoplasm. No male sterility was observed attributable to ms_3 in RN613. Averaged over environments, RN613 was 7 d later in maturity (88 d vs. 81 d) and 5 cm taller in height (129 cm vs. 124 cm) than RTx430 (Table 3). Seedling emergence of RN613 was significantly lower than for RTx430. Grain yield of RN613 was significantly lower (5.7 t ha^{-1} vs. 6.7 t ha^{-1}) compared with RTx430, but residue yield of RN613 was significantly higher (7.1 t ha^{-1} vs. 6.6 t ha^{-1}) compared with RTx430. Neutral detergent fiber of RN613 was equivalent to that of RTx430, while ADF was slightly lower. Acid detergent lignin was significantly lower in RN613 (44 g kg^{-1}) compared with N609 (RTx430 *bmr*-6, 57 g kg^{-1}), and both were lower in ADL than wild-type RTx430 (66 g kg^{-1}). RN613 ADL content was not significantly different from N610 (RTx430 *bmr*-12, 49 g kg^{-1}). Complete comparisons of RN613 with N609, N610, and wild-type RTx430 are shown in Table 3.

Table 1. Characteristics of BN611 sorghum genetic stocks and comparisons with other Atlas-derived lines near-isogenic for single *bmr* genes or wild-type.

Characteristic	BN611 (*bmr*-6 + *bmr*-12)	N598 (*bmr*-6)	Atlas *bmr*-12 (***bmr***-12)	Atlas (wild-type)	SE
Emergence (plants m^{-1})	16.2 a†	14.1 c	15.8 ab	15.7 b	0.4
50% anthesis (d)	83 a	78 b	82 a	79 b	1
Height (cm)	214 d	241 c	253 b	278 a	9
Biomass yield (t ha^{-1})	10.5 c	15.7 b	14.8 b	17.7 a	4.6
NDF‡ (g kg^{-1})	550 a	522 b	526 b	531 ab	5
ADF (g kg^{-1})	302 a	294 b	292 b	307 a	5
ADL (g kg^{-1})	35 c	45 b	41 b	54 a	6

†Means in rows followed by a different letter differ at $P = 0.05$ using the DIFF option of PROC MIXED (SAS Institute, Cary, NC).

‡NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table 2. Characteristics of BN612 sorghum genetic stocks and comparisons with other Wheatland-derived lines near-isogenic for single *bmr* genes or wild-type.

Characteristic	BN612 (*bmr*-6 + *bmr*-12)	N599 (*bmr*-6)	N600 (*bmr*-12)	Wheatland (wild-type)	SE
Emergence (plants m^{-1})	13.2 b†	13.6 ab	13.6 ab	14.2 a	8.3
50% anthesis (d)	80 a	77 b	78 a	76 b	1
Height (cm)	98 b	91 c	105 a	104 a	1
Grain yield (t ha^{-1})	5.6	6.2	5.3	6.3	1.2
Residue yield (t ha^{-1})	4.6 b	4.4 c	5.1 a	5.2 a	0.2
NDF‡ (g kg^{-1})	664 b	660 b	671 b	685 a	10
ADF (g kg^{-1})	370 b	369 b	373 b	394 a	10
ADL (g kg^{-1})	47 b	55 b	48 b	64 a	7

†Means in rows followed by a different letter differ at $P = 0.05$ using the DIFF option of PROC MIXED (SAS Institute, Cary, NC).

‡NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

Table 3. Characteristics of RN613 sorghum genetic stocks and comparisons with RTx430 other derived lines near-isogenic for single *bmr* genes or wild-type.

Characteristic	RN613 (*bmr*-6 + *bmr*-12)	N609 (*bmr*-6)	N610 (*bmr*-12)	RTx430 (wild-type)	SE
Emergence (plants m^{-1})	9.3 b†	9.5 b	9.4 b	12.2 a	0.6
50% anthesis (d)	88 a	82 b	89 a	81 b	4
Height (cm)	129 b	119 d	134 a	124 c	5
Grain yield (t ha^{-1})	5.7 b	6.5 a	5.4 b	6.7 a	0.6
Residue (t ha^{-1})	7.1 a	5.4 c	7.0 a	6.6 b	0.2
NDF‡ (g kg^{-1})	675 b	694 a	694 a	687 ab	18
ADF (g kg^{-1})	373 c	396 ab	386 b	397 a	6
ADL (g kg^{-1})	44 c	57 b	49 c	66 a	11

†Means in rows followed by a different letter differ at $P = 0.05$ using the DIFF option of PROC MIXED (SAS Institute, Cary, NC).

‡NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin.

These results confirm our earlier observations that genetic background effects influence the expression of *bmr* genes (Oliver et al., 2005a,b) and extend those to possible relationships among *bmr* genes, other genes, and genetic background. For example, in all three backgrounds, time to 50% anthesis of the double mutant is the same as that of the *bmr*-12 mutant, suggesting that the *bmr*-6 mutation does not counteract the delay in maturity caused by the *bmr*-12 mutation. Conversely, in the grain-type 3-dwarf backgrounds, height of the double mutant was intermediate between the two single mutants, while in the taller forage-type 1-dwarf background the height of the double mutant was less than either of the single mutants, suggesting possible differing epistatic interactions among *bmr* and dwarfing genes. From a utilization point of view, it is also important to note that total biomass yield in the forage background was greatly reduced in the double mutant compared with the single mutants and wild-type Atlas.

When crossed to various *bmr*-6 or *bmr*-12 testers, BN611, AN612, BN612, and RN613 had brown midrib progeny indicating that both *bmr* genes are present and stable in these four genetic stocks (Table 4). Fertility restoration in selfed F_1 progeny of these same crosses confirm A-, B-, and R- reactions as indicated by the genetic stock prefixes (Table 4).

Table 4. Midrib color and fertility of progeny of N611, N612, and N613 sorghum genetic stock test crosses to verify presence of both *bmr*-6 and *bmr*-12, and expected fertility restoration reaction.

Seed parent	Pollen parent	Midrib color of F_1 progeny		Fertility of selfed F_1 progeny	
		No. brown individuals	No. wild-type individuals	No. sterile individuals	No. fertile individuals
AN599 (Wheatland *bmr*-6)	BN611 (Atlas *bmr*-6 *bmr*-12)	30†	0	6	0
AN600 (Wheatland *bmr*-12)	BN611 (Atlas *bmr*-6 *bmr*-12)	31	0	6	0
AN612 (Wheatland *bmr*-6 *bmr*-12)	BN599 (Wheatland *bmr*-6)	30	0	6	0
AN612 (Wheatland *bmr*-6 *bmr*-12)	BN600 (Wheatland *bmr*-12)	30	0	6	0
AN612 (Wheatland *bmr*-6 *bmr*-12)	BWheatland	0	30	6	0
AN599 (Wheatland *bmr*-6)	BN612 (Wheatland *bmr*-6 *bmr*-12)	30	0	6	0
AN600Wheatland *bmr*-12)	BN612 (Wheatland *bmr*-6 *bmr*-12)	27	0	6	0
AN599 (Wheatland *bmr*-6)	N613 (RTx430 *bmr*-6 *bmr*-12)	31	0	0	6
AN600 (Wheatland *bmr*-12)	N613 (RTx430 *bmr*-6 *bmr*-12)	30	0	0	6

†The probability that test cross expectations were met exceed $P = 0.9999$ using χ^2 test for goodness of fit for all test crosses.

Availability

Seed of these genetic stocks is maintained and distributed by the USDA-ARS, Grain, Forage, and Bioenergy Research Unit, Department of Agronomy, University of Nebraska, Lincoln, NE 68583-0737, and will be provided without cost to each applicant on written request. Genetic material of this release will be deposited in the National Plant Germplasm System where it will be available for research purposes, including development and commercialization of new cultivars. It is requested that appropriate recognition be made if these genetic stocks contribute to the development of a new breeding line or cultivar.

References

Bittinger, T.S., R.P. Cantrell, and J.D. Axtell. 1981. Allelism tests of the brown midrib mutants of sorghum. J. Hered. 72:147–148.

Bout, S., and W. Vermerris. 2003. A candidate-gene approach to clone the sorghum brown midrib gene encoding caffeic acid O-methyltransferase. Mol. Gen. Genet. 269:205–214.

Brown, H.B., J.A. Clark, E.F. Gaines, H.K. Hayes, W.J. Morse, J.H. Parker, T.R. Stanton, G.H. Stringfield, and M.A. McCall. 1936. Varietal standardization and registration. J. Am. Soc. Agron. 28:1027.

Bucholtz, D.L., R.P. Cantrell, J.D. Axtell, and V.L. Lechtenberg. 1980. Lignin biochemistry of normal and brown midrib mutant sorghum. J. Agric. Food Chem. 28:1239–1245.

Miller, F.R. 1984. Registration of RTx430 sorghum parental line. Crop Sci. 24:1224.

Oliver, A.L., J.F. Pedersen, R.J. Grant, and T.J. Klopfenstein. 2005a. Comparative effects of the sorghum *bmr*-6 and *bmr*-12 genes: I. Forage sorghum yield and quality. Crop Sci. 45:2234–2239.

Oliver, A.L., J.F. Pedersen, R.J. Grant, T.J. Klopfenstein, and H.D. Jose. 2005b. Comparative effects of the sorghum *bmr*-6 and *bmr*-12 genes: II. Grain yield, stover yield, and stover quality in grain sorghum. Crop Sci. 45:2240–2245.

Pedersen, J.F., D.L. Funnell, J.J. Toy, A.L. Oliver, and R.J. Grant. 2006a. Registration of 'Atlas *bmr*-12' forage sorghum. Crop Sci. 46:478.

Pedersen, J.F., D.L. Funnell, J.J. Toy, A.L. Oliver, and R.J. Grant. 2006b. Registration of seven forage sorghum genetic stocks near-isogenic for the brown midrib genes *bmr*-6 and *bmr*-12. Crop Sci. 46:490–491.

Pedersen, J.F., D.L. Funnell, J.J. Toy, A.L. Oliver, and R.J. Grant. 2006c. Registration of 12 grain sorghum genetic stocks near-isogenic for the brown midrib genes *bmr*-6 and *bmr*-12. Crop Sci. 46:491–492.

Pedersen, J.F., and K.J. Moore. 1995. An automated plot harvest system for use with a commercial forage harvester. Agron. J. 87:605–607.

Porter, K.S., J.D. Axtell, V.L. Lechtenberg, and V.F. Colenbrander. 1978. Phenotype, fiber composition, and in vitro dry matter disappearance of chemically induced brown midrib (*bmr*) mutants of sorghum. Crop Sci. 18:205–208.

Sarath, G., R.B. Mitchell, S.E. Sattler, D. Funnell, J.F. Pedersen, R.A. Graybosch, and K.P. Vogel. 2008. Opportunities and roadblocks in utilizing forages and small grains for liquid fuels. J. Ind. Microbiol. Biotechnol. 35:343–354.

SAS Institute. 2000–2004. SAS 9.1.3: Help and documentation. SAS Inst., Cary, NC.

Vogel, K.P., J.F. Pedersen, S.D. Masterson, and J.J. Toy. 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39:276–279.